# Expression of Epithelial Mucins MUC1, MUC2, and MUC3 in Ductal Carcinoma In Situ of the Breast

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■ **Abstract:** Epithelial mucins are glycoproteins secreted by epithelial cells and their carcinomas. At least nine mucin genes have been identified, and their products (MUC1–MUC9) are expressed in various epithelia. MUC1 is a mucin expressed in breast epithelial cells, whereas MUC2 and MUC3 are primarily intestinal mucins. Although MUC1 and MUC2 expression has been documented in invasive ductal carcinoma of the breast, mucin expression in pure ductal carcinoma in situ (DCIS) has not been investigated. Sixty-one of 105 cases of DCIS without coexisting infiltrating carcinoma diagnosed during a 30-month period were selected as having sufficient tissue for study. Paraffin-embedded tissue sections were stained using immunohistochemical techniques with mouse monoclonal anti-MUC1, anti-MUC2, and rabbit-specific polyclonal anti-MUC3 antibodies. Immunoreactive epitopes of MUC1, MUC2, and MUC3 were expressed in DCIS in 61, 19, and 16 of 61 cases, respectively. MUC2 and MUC3 staining intensity in DCIS was markedly less than that observed for MUC1. Luminal and/or cytoplasmic patterns of staining were observed for MUC1. MUC2 and MUC3 showed only cytoplasmic staining. Cytoplasmic-only staining of MUC1 was associated with a higher grade of DCIS. Any MUC2 staining was also associated with a higher grade of DCIS. Coexpression of MUC2 and MUC3 was present in only 6 of 61 cases, and MUC3 staining was unrelated to the grade of DCIS. Cytoplasmic expression of MUC1 and MUC2 appears to be associated with a higher grade of DCIS. MUC3 expression appears to be independent of grade and expression of MUC1 and MUC2. The relationship of mucin expression and grade warrants further study. ■

**Key Words:** breast, ductal carcinoma in situ, epithelial membrane antigen, immunohistochemistry, mucin

Epithelial mucins are transmembrane glycoproteins that are produced by both normal epithelial cells and malignant epithelial tumors of pulmonary, gastrointestinal, gynecologic, and mammary origin. Mucins are complex molecules ranging in size from 400 kDa to more than 1,000 kDa. They are a heterogeneous group of molecules whose variations in molecular structure are thought to carry tissue-specific functions (1–4). Nine such mucins, MUC1–MUC9, have had their genes identified and their respective products either completely or partially characterized (5,6). All mucins are characterized by a tandemly paired and repetitive central peptide that is rich in serine and threonine. The peptides have little to no homology among mucin types and are thus ideal epitopes for raising type-specific antibodies to mucins (2,4,7). MUC1, also known as epithelial membrane antigen, is the most extensively studied of the mucins (8). Although it was originally described as a tissue-specific glycoprotein of breast epithelium, MUC1 is present in most polarized epithelial cells (7–12). The expression of other secretory mucin may be localized to specific tissue types. MUC2 and MUC3 are thought to be prima-

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rily expressed in gastrointestinal tissues. MUC4, MUC5, and MUC8 are found in bronchial tissues. MUC7 is found in salivary gland and MUC6 in gastric epithelium (13–17). MUC2, MUC3, MUC5, and MUC6 have highly conserved cysteine domains which are thought to form disulfide bonds between mucin monomers, accounting for the high viscosity of these mucins (18). The overall tissue distribution of mucins in normal and malignant epithelial cells has not been completely elucidated (13–16).

Likewise the patterns of mucin expression in benign and malignant breast tissue have not been completely established. MUC1 is the only mucin that characteristically is expressed by normal breast epithelium (5,8,19). Several studies have focused on MUC1, MUC2, and MUC5 expression in invasive carcinomas of the breast (13,16–18) and have noted expression of these mucins in coexisting normal and atypical duct epithelium and DCIS (17,18). MUC1 has been shown to be overexpressed in invasive ductal carcinoma and is thought to facilitate metastatic behavior (8). MUC2 and MUC5 are found in colloid carcinomas of the breast and are variably present in adjacent benign epithelium (11,16,18). MUC2 has been shown to be absent in most nonmucinous ductal carcinomas of the breast. Overexpression of MUC2 by infiltrating ductal carcinomas has been associated with more aggressive behavior than those without (17). The preferred site of expression for MUC3 is the absorptive cell of the small intestine (6). The presence of MUC3 in normal breast epithelium has not been reported.

Mucin expression in DCIS without concurrent invasive disease (so-called pure DCIS) has not been previously studied. Characterizing the expression of different epithelial mucins in DCIS would help to determine a baseline rate of mucin expression and delineate the differences between normal ducal epithelial cells and those that form this important precursor lesion of invasive breast carcinoma. The primary focus of this study is to compare the expression of MUC1 with MUC2 and MUC3 in cases of pure DCIS, where no invasive carcinoma exists, and the adjacent nonmalignant breast epithelium.

## MATERIALS AND METHODS

Clinical histories and slides from consecutive patients with a diagnosis of DCIS were reviewed. Cases with insufficient in situ tumor for study and cases with coexisting infiltrating carcinoma were excluded; 105 cases were found to meet the above requirements. From each case, two blocks containing DCIS were sectioned at 3 μm intervals and mounted on positively charged glass slides for a total of 12 unstained slides. The first and last sections were stained with hematoxylin and eosin to confirm the presence of lesional tissue. Forty-four cases did not have lesional DCIS maintained in the cut sections and these were excluded from the study. The intervening sections from the remaining 61 cases were stained for MUC1, MUC2, and MUC3.

**Table 1. MUC1, MUC2, and MUC3 Staining Patterns for 61 Cases of Pure DCIS**

| Staining pattern | MUC1 | MUC2 | MUC3 |
|---|---|---|---|
| None | 0 | 42 | 45 |
| Cytoplasmic only | 26 | 19 | 16 |
| Combined | 26 | 0 | 0 |
| Luminal only | 9 | 0 | 0 |

The immunohistochemical method is as follows: Unstained sections were deparaffinized and hydrated using graded xylene and alcohol solutions. Sections were stained using an automated stainer with an avidin-biotin peroxidase system. Sections were incubated with mouse anti-MUC1 (clone B24.1, Biomeda Corp., Foster City, CA), mouse anti-MUC2 (clone B306.1, Biomeda), and rabbit polyclonal anti-MUC3 (Biomeda). Staining dilutions were 0.01 μg/ml for MUC1, 0.01 μg/ml for MUC2, and 0.02 μg/ml for MUC3. Negative controls used nonimmune serum on four cases. Tissue sections of stomach and breast were used as controls as suggested by the manufacturer.

Stained sections were reviewed by two authors for type, pattern, and amount of mucin positivity. Sections were scored for percentage of carcinoma in situ cell staining and the location of staining within positive cells. Cells were considered staining positive if the brown pigment of diaminobenzidine could be readily detected at scanning magnification. The locations of staining consisted of luminal (staining of the duct luminal surface), membranous (staining of the entire cyto-

**Table 2. MUC1 Staining Compared to Grade of DCIS**

| | Grade of DCIS | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | Total |
| Luminal only | 3 | 6 | 0 | 9 |
| Combined luminal cytoplasmic | 8 | 11 | 7 | 26 |
| Cytoplasmic only | 1 | 6 | 19 | 26 |
| Total for grade | 12 | 23 | 26 | 61 |

plasmic border), and cytoplasmic (granular staining of cell cytoplasm). The percentage of DCIS or epithelial cells staining was scored as 0, focal, and positive as follows: 0 (0% cells staining), focal (any cells up to 5% of tumor cells), and positive (greater than 5% of tumor cells). Tumor cells were compared to the epithelial staining present in adjacent benign breast tissue. Results were compared using Prophet (BBN Systems and Technologies), a statistics program sponsored by the National Institutes of Health, using chi-squared 2 × 2-test.

## RESULTS

Of the 61 cases of DCIS, 12 were grade 1 and were predominately cribriform or solid patterns. Twenty-four cases were grade 2 and were cribriform or solid with necrosis. The remaining 25 DCIS cases were grade 3 and were predominately solid with comedo-type necrosis.

Table 1 summarizes the number of DCIS cases staining for each mucin type and the pattern of staining. Sixty of 61 cases (98%) demonstrated positive staining for MUC1 and 1 case was focally positive. Twenty-six cases (40%) had cytoplasmic-only staining, 9 cases (15%) showed only luminal staining, and 26 cases (40%) had combined cytoplasmic and membranous staining. Benign breast epithelium in 42 of 61 cases showed a luminal-only pattern of staining for MUC1. MUC1 staining was less intense in the benign epithelium of the same case when compared to staining of DCIS for the same case. Table 2 shows the distribution of MUC1 staining compared to the grade of DCIS. Nineteen of 26 grade 3 DCIS cases had only cytoplasmic staining for MUC1 compared to 7 of 35 grade 1 and 2 cases ($p = 0.002$).

Nineteen cases of DCIS (32%) stained with antibody to MUC2 and all of these cases demonstrated a cytoplasmic pattern of staining. Of these, 8 had only focal staining and 11 were considered positive with more than 5% of tumor cells staining. Benign breast epithelium in two cases had focal staining for MUC2; the remaining 59 showed no staining with anti-MUC2 antibody. Both cases with staining of benign epithelium had coexisting MUC2-positive in situ lesions. Compared to staining for MUC1, MUC2 staining of tumor cells was observed to be less intense than that seen for MUC1 staining of DCIS. Table 3 shows the distribution of staining for MUC2 compared to the grade of DCIS. Twelve of the 19 cases with any staining for MUC2 were grade 3 in situ carcinomas ($p < 0.006$).

Sixteen cases of DCIS stained with polyclonal antibody to MUC3; of these 9 cases had only focal staining of tumor cells and 7 cases had greater than 5% of tumor cells staining. All 16 cases demonstrated a cytoplasmic pattern of staining. Adjacent benign breast epithelium demonstrated faint (background quality) staining in 53 cases in a cytoplasmic in pattern; the optical intensity was insufficient for positive staining. The distribution of staining for MUC3 compared to DCIS grade is shown in Table 4. A correlation with MUC3 positivity and high grade was not observed.

Table 5 lists the distribution of staining for MUC2 compared to MUC3. Most cases that had staining for MUC2 were negative for MUC3. Twenty-nine cases had staining for MUC2 and/or MUC3, but only six cases showed staining for both mucins, with one or both mucins showing only focal staining.

## DISCUSSION

The results of this study establish a rate of expression in pure DCIS for three mucins: MUC1, MUC2, and MUC3. MUC1 staining was observed in every case of DCIS (Fig. 1) and was expressed strongly in all but a single case. Three staining patterns were present for MUC1:

**Table 3. Staining for MUC2 Compared to Grade of DCIS**

| | Grade of DCIS | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | Total |
| No staining for MUC2 | 9 | 20 | 13 | 42 |
| Focal positive MUC2 | 0 | 2 | 6 | 8 |
| Positive MUC2 | 3 | 2 | 6 | 11 |
| Total for grade | 12 | 24 | 25 | 61 |

**Table 4. Staining for MUC3 Compared to Grade of DCIS**

| | Grade of DCIS | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | Total |
| No MUC3 staining | 10 | 16 | 19 | 45 |
| Focal MUC3 staining | 1 | 3 | 5 | 9 |
| Positive MUC3 staining | 1 | 5 | 1 | 7 |
| Total for grades | 12 | 24 | 25 | 61 |

**Table 5. Staining of MUC2 Compared to MUC3 for 61 Cases of DCIS**

| | Staining for MUC2 | | | |
|---|---|---|---|---|
| | Negative | Focal | Positive | Total |
| MUC3 negative | 32 | 5 | 8 | 45 |
| Focal MUC3 | 4 | 2 | 3 | 9 |
| Positive MUC3 | 6 | 1 | 0 | 7 |
| Total | 42 | 8 | 11 | 61 |

Figure 1. DCIS stained with monoclonal antibody to mucin type 1 (MUC1); staining is present in both the cytoplasm of the in situ carcinoma lining the ducts and variably at the luminal surface of the ducts. (Diaminobezidine with hematoxylin counterstain; original magnification ×200.)

Figure 2. DCIS stained with monoclonal antibody to mucin type 2 (MUC2); variable granular cytoplasmic staining is present in many of the cells. (Diaminobezidine with hematoxylin counterstain; original magnification ×200.)

luminal only, cytoplasmic only, and combined luminal cytoplasmic. MUC1 is the only mucin currently described as a cytoplasmic-membrane bound molecule and this property explains the observed luminal position of staining in the cases studied (5,8). MUC1 expression was present not only in DCIS but also in adjacent benign breast epithelium. Stronger MUC1 staining was generally seen in DCIS compared to the adjacent benign epithelium. This pattern of MUC1 expression is similar to that described for infiltrating carcinomas (8,9). Also the loss of luminal expression of MUC1 with only cytoplasmic MUC1 was associated with higher-grade DCIS. This benign and neoplastic staining pattern for MUC1 parallels that recently described in the pancreas by Monges et al. (19) where primarily apical (luminal) staining is expressed in benign pancreatic acini compared to cytoplasmic MUC1 expression present in ductal adenocarcinomas.

MUC2 expression (Fig. 2) was observed in only 28% (19 of 61) of cases of DCIS. MUC2 is characterized as a gel-forming protein and this correlated with a purely cytoplasmic staining location of this mucin that was observed in this study. Expression of MUC2 in adjacent benign breast tissue was weak to absent, suggesting that MUC2 is relatively DCIS specific when positive staining is detected. Like cytoplasmic expression of MUC1, MUC2 expression in DCIS was strongly associated with higher grade. Although MUC2 has been shown to be strongly expressed by invasive colloid carcinoma, a low-grade tumor in breast (16,18), MUC2 positivity has been correlated with more aggressive tumor behavior and poorer prognosis in infiltrating ductal carcinomas when compared to MUC2-negative tumors. The association of MUC2 expression with higher-grade DCIS would parallel this.

This is the first study that the authors are aware of which describes MUC3 expression in breast epithelium. We found that the pattern of staining for MUC3 in DCIS and benign breast epithelium was similar to that of MUC2 (Fig. 3). However, only 6 of 61 cases showed coexpression of MUC2 and MUC3. MUC3 expression did not show a correlation with the grade of DCIS as was found for MUC2 and MUC1.

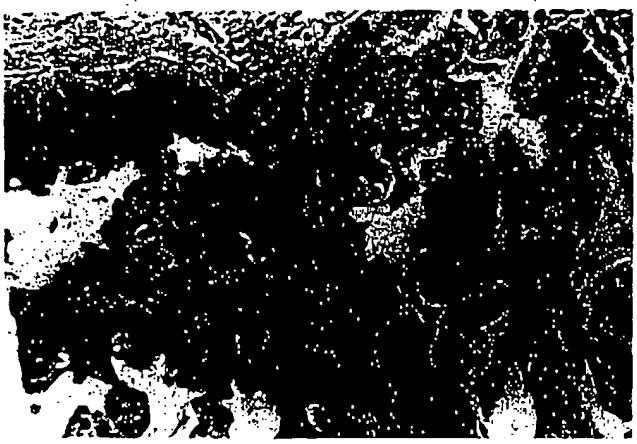

Figure 3. Polyclonal antibody raised against mucin type 3 is used to stain cells of DCIS. Scattered cells show heavy cytoplasmic staining, sharply defining them against adjacent negatively staining cells. (Diaminobezidine with hematoxylin counterstain; original magnification ×330.)

This study, using cases of pure DCIS, showed that DCIS has patterns of MUC1 and MUC2 expression similar to those described for invasive carcinoma. Of interest is the observation that MUC2 is expressed in 19 of 61 (31%) DCIS cases studied and in benign tissue in only 2 of 61 cases. This suggests that expression of this mucin is a marker of neoplasia in breast tissue. Cytoplasmic expression of MUC1 also appears to mark neoplastic change in DCIS, in that the mechanism of membrane attachment of MUC1 appears to be absent or defective in a portion of in situ carcinomas. Immunoreactive MUC3 expression of neoplastic breast epithelium using polyclonal antibody does not appear to correlate with MUC2 expression, MUC1 cytoplasmic expression, or grade of DCIS. Its expression was in a minority of DCIS cases in this study, and whether it is expressed in invasive carcinoma needs to be further investigated, preferably using monoclonal antibodies as they become available for study.

Potential clinical applications utilizing specific mucin epitopes of neoplastic breast diseases range from diagnostic to therapeutic. CA 15-3 (MUC1) is currently used to monitor response to breast cancer therapy. Radiolabeled anticancer antibodies against breast-specific mucins may be utilized for high-resolution imaging as well as tumor-directed therapy, similar to antibody against HER-2/*neu* (20,21). Mucin protein and carbohydrate epitopes are strong candidates for a tumor vaccine that may someday target breast cancer cells specifically.

MUC1 overexpression and MUC2 expression are found to occur in higher-grade in situ lesions. Kanthan et al. (22) recently noted that aberrant mucin expression may be an early step in oncogenesis. These findings and those of this study serve to validate the use of grade as a marker of biologic behavior. The differential mucin expressions are worthy of further investigation to determine if they will prospectively allow the identification of duct carcinoma lesions at risk for invasion or recurrence.

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